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The effect of European Red Mite Feeding Injury on certain metabolic activities of Red Delicious apple leaves

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SUMMARY

The results of this investigation substantiate, with experimental evidence, the belief that the feeding injury of European red mite, *Metatetranychus ulmi*, is detrimental to the metabolic activities of apple foliage. Although this study must be regarded as an individual and specific situation because of the unavoidably unnatural conditions imposed on both the trees and mites in the greenhouse, enough information of a relative nature may be drawn from the data to project conclusions applicable to orchard conditions.

There are strong indications that severe injury is primarily a function of time. The number of mites per leaf is of importance but what apparently is more significant is the time at which these numbers are found. Twenty mites per leaf in late July would be of far less concern than three or perhaps fewer mites per leaf in May. In the latter case the build-up of the mite population, resulting in a reduced rate of carbohydrate production by the foliage, would come at about the time fruit bud differentiation is taking place. A later build-up would possibly interfere with late storage of food reserves. In addition, Chapman, Lienk and Curtis (4) state that leaves injured in midseason did not "recover" and exhibited a low chlorophyll content in late season. These authors state further that they had observed in the past what appeared to be a partial recovery of leaf function as evidenced by leaf color. Perhaps the severity of the damage determines whether this recovery can take place or whether the leaf will remain depleted of chlorophyll for the remainder of the season. As shown in this study there is no reason to believe that severely injured leaves will exhibit other than a lowered rate of photosynthesis during the remainder of the season. Assuming that all else is equal, it follows that a tree with foliage injured early in the season will enter dormancy in a less thrifty condition than one suffering later injury.

Results of the investigation also disclose that the feeding of the European red mite causes a serious imbalance in the transpiration rates of the injured leaves. Rates as low as those encountered may be due to damage to the stomatal cells, inflicted when the mite feeds on the lower surfaces of the leaves.

As indicated earlier, a reduction in the chlorophyll content of injured leaves may take place before photosynthesis and transpiration of these leaves are affected. In the writer's opinion, this is of value in that it affords the observer time to detect a rising

infestation before apparent physiological injury occurs. However, since the decrease in photosynthesis and transpiration is rapid beyond this point, necessary control measures should not be delayed.

There is no question of the advisability of the use of ovicidal materials if overwintering eggs are found on the trees, or the application of acaricidal materials if mites are present on the foliage in early season. Exceptions may be those in which natural control is known to be effective, or if other materials not specifically acaricidal but effective none the less are used for the control of other pests in the routine spray applications. In such cases, careful observations will indicate whether these alternate measures are effective, and corrective steps may be taken if they are found to be wanting in degree of control.

BULLETIN 570

THE EFFECT OF EUROPEAN RED MITE FEEDING INJURY ON CERTAIN METABOLIC ACTIVITIES OF RED DELICIOUS APPLE LEAVES[1]

L. W. BOULANGER[2]

INTRODUCTION

Numerous references may be found dealing with the damage caused to deciduous fruit trees by the feeding of the European red mite, *Metatetranychus ulmi* (Koch). There is a general belief, founded on many observations, that trees so injured suffer a reduction in the rate of productivity. However, a review of the literature reveals that very little experimental evidence can be found to support this assumption.

Mature trees undoubtedly have a reserve of food materials ample enough to produce a crop during a year of severe mite injury, and in such cases the effect of the injury may not be evident except as actual damage to the foliage. However, the nonevident portion of the injury may be more detrimental than might be supposed. Lowering of tree vigor, making them more susceptible to winter injury and reduction of bud formation which may reduce the next year's crop are delayed effects which may not be apparent for some time after injury occurs.

The results of an investigation of the effect of such feeding injury on certain metabolic activities of Red Delicious leaves are presented in this bulletin.

MEASURING THE EFFECT OF THE INJURY

It is clear that any quantitative measurement of the effect of feeding injury cannot be based on the tangibles commonly associated with insect damage and must start at a more fundamental stage in the complex biochemical process leading to the production of the fruit. Since the mite feeds by sucking out the contents of the leaf cells, it must also necessarily remove chlorophyll which is essential to photosynthesis. There are various methods available which can be used to determine what is called "the apparent rate of photosynthesis." Leaf injury is a gradual process dependent upon the number of mites feeding and the length of time they

[1] Presented to the faculty of the Graduate School of Cornell University as a major thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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are allowed to do so. As these numbers do not increase to harmful levels until the third or fourth generation, it is necessary that the leaves be kept in good condition and that the mites thereon be not overly disturbed during the measurements.

The method best meeting the requirements of the study was that developed by Heinicke and Hoffman (11 and 12).[3] It was subsequently used in a study similar to this one by Marshall, Childers and Brody (18) who employed it to determine the effect of leafhopper feeding on the apparent rate of photosynthesis of apple leaves. Essentially, the apparatus is a closed system through which an airstream is drawn, first passing over a definite area of leaf surface limited by a glass cup, then through an absorption tower containing a carbon dioxide absorbing solution, and through a flowmeter. A vacuum pump is used to exhaust the tower thereby creating the airstream. The absorbing solution is introduced into the system in a reservoir flask which is connected between the leaf cup and the tower. A wet test meter is used to calibrate the flowmeter during operation. Except for minor modifications, the apparatus used in this study was essentially the same as that described and used by the above authors. The apparent rate of photosynthesis as determined in this study will be referred to simply as photosynthesis.

Provisions were also made to determine the effect of the feeding injury on the respiration, transpiration, and chlorophyll content of the damaged leaves.

NATURE OF THE INJURY

Physical Injury. Papers dealing with gross damage are numerous and no attempt will be made to cover the subject in its entirety. The reader is referred to the synopsis of world literature on *M. ulmi* by Groves (9) which has an extensive bibliography on such works.

Blair (1), in what is perhaps the most comprehensive paper to date on the injury to the leaf, states that when the pest is feeding on the under surface, its mouthparts penetrate the epidermis, pass through the spongy mesophyll, and reach the palisade layers where the cell contents are sucked out. The chloroplasts disappear and the small amount of remaining cellular material coagulates to form an amber mass at one end of the cell. In the palisade layers, only the penetrated cells are damaged; adjacent cells show no evidence of injury. In very badly damaged leaves, the entire contents of these layers may be removed. It also appears that no attempt is made by the mite to penetrate the vascular bundles.

Blair's findings confirmed the work of Lewis (Groves, 9). These workers observed that in most cases, triploid and tetraploid varieties suf-

[3] Refers to literature cited, page 33.

fered less damage than did diploid varieties. They theorized that this was due to the thinner palisade layers in the diploid varieties.

Physiological Injury. Agreement can be found in the papers of many workers in which the physiological effects of mite feeding are surveyed. Their conclusions are that the range of injury includes defoliation, fruit that is unripe, sour, undersized, poorly colored and prone to early dropping, a reduction in the number of fruit buds set for the following season, and depletion of stored food, leading to a susceptibility to frost and winter injury (de Ong, 7; Bourne, 2; Herrick, 13; Hammer, 10).

Chapman, Lienk and Curtis (4) published tentative conclusions based on results of the first growing season in a long-term study of the effect of unchecked mite infestations on three varieties of apple; Cortland, Red Delicious, and Rome. They found that uninfested trees, as compared to infested trees, had a greater increase in trunk girth and produced a greater number of fruits, although no appreciable difference in individual fruit size was noted. They also found that the number of fruits set on marked Cortland branches which were kept free of mites was as much as 10 per cent greater than that on injured branches of the same variety. This last suggests, according to the authors, that the yield decrease of injured trees is the result of lowered fruit set. They did not find, however, any difference between fruit from injured trees and fruit from uninjured trees with respect to firmness, either before or after storage. There was a similar lack of difference with respect to soluble solids measured at harvest. Contrary to previous reports (Lathrop,15; Groves, 9) fruits from the injured trees were better colored than were those from mite-free trees. No observable leaf drop resulted from mite feeding as such.

Further studies by Lienk, Chapman and Curtis (16) indicated that unchecked mite feeding for one season caused a 75 per cent and 34 per cent reduction in bloom the following year in Cortland and Red Delicious, respectively. Yields in bushels of fruit at harvest were 64.8 per cent and 35.8 per cent lower, respectively. The writers report that there was little visible indication of damage in Cortland trees but that feeding injury was clearly evident in Red Delicious trees. Cortland leaf injury consisted only of a uniform paling of the leaves although the effect was considerable on fruit bud formation.

INDUCING THE INJURY UNDER GREENHOUSE CONDITIONS

Test trees and mite cultures. Red Delicious trees were used in the study. These were grown in earthenware pots and were cut back to a point about 5 inches above the bud union. One shoot was allowed to

develop from the stub, and daily watering and bimonthly applications of nutrient solutions resulted in vigorous growth. Shoots attained a height of about 5 feet and leaves often exceeded 5 inches in length. Mite cultures were established on trees not suited for experimental purposes, and these cultures provided an adequate supply of mites for testing.

Establishing the ratio between check and test leaves. Before controlled infestations were set out on the test leaves, the differences in photosynthesis, respiration, and transpiration between a check leaf and an adjacent test leaf of equal size and vigor on the same shoot were determined and ratios were established. Ratios were established for three replicates, each on a separate tree, giving a total of six leaves for each of the six population groups described below. Four photosynthesis determinations and one respiratory determination were conducted, covering 2 days and 1 night. Each determination run was of 4 hours duration. The ratio obtained was then used as a basis for measuring the reduction in the expected rate of photosynthesis and respiration in subsequent determinations, according to the method of Marshall, Childers and Brody (18).

In addition to the 36 leaves to be used in the photosynthesis and respiration determinations, 18 trees were selected to be used in the chlorophyll study. Twelve closely paired leaves at about the middle of the shoots were tagged on each tree. Check and test leaves were alternated on the shoots and each population group was replicated on two other trees. This procedure was repeated six times, making possible three separate samples for each chlorophyll determination with each sample being taken from a different tree.

Establishing experimental infestations. Populations were set out on the test leaves under two separate plans. Series 1 was designed to show the effect of a small initial population over an extended period of time. Series 2 was designed to show the damage caused by a high population in a shorter period.

The following numbers of mites were transferred: Series 1: group 1, 3 adult females; group 2, 5 adult females; group 3, 10 adult females; group 4, 20 adult females. Series 2: group 1, 50 mites; group 2, 100 mites.

On the day following the ratio determinations, all test leaves of the group so established were infested with the number of mites listed above. Mites in series 1 were transferred individually by means of a small camel's hair brush trimmed of all but a few of its bristles. In series 2, the mites were counted with a low-power binocular dissecting microscope and entire leaves or leaf sections bearing the required numbers of mites were pinned to the test leaves and allowd to dry out thus inducing a

migration. Sexes were indiscriminately transferred in this series.

A pipe cleaner was wrapped around the petiole of each infested leaf and covered with petroleum jelly to keep migration at a minimum. In all but a few cases this barrier proved to be adequate in retaining the mites on the required leaves.

DETERMINATION OF THE INJURY

Effect on photosynthesis and respiration. The filling of the reservoir flasks with the carbon dioxide-absorbing solution was done in the laboratory prior to a run. One hundred cc of approximately 0.2N NaOH were run into the flasks from a pipette and 10 drops of n-butyl alcohol were added to decrease the surface tension, as suggested by Thomas (20). One hundred cc of distilled water were added to complete the charge. The flasks were closed until connected to the towers. The pinchcocks were then removed and the vacuum pump started. The liquid moved up and out of the flask into the tower, and the time was recorded at the moment the airstream began bubbling through the solution. Air flow was maintained at between 1.5 and 1.75 cubic feet per hour per cup.

When the required time had elapsed, the pump was turned off and the rubber stoppers at the tops of the towers removed, allowing the solutions to return to the flasks. When empty, the towers were rinsed thoroughly with at least 200 cc of distilled water each and the flasks closed after this had drained from the towers. The flasks were then returned to the laboratory where 10 cc of 25 per cent barium chloride solution was added to each. They were then brought to volume with distilled water, shaken vigorously, and set aside.

When the barium carbonate precipitate had settled, 100 cc of the clear solution were pipetted out and titrated against approximately 0.10N hydrochloric acid with phenolphthalein indicator. The titration readings were then converted to milligrams of carbon dioxide absorbed per 100 square centimeters of leaf surface by means of the formulae given by Heinicke and Hoffman (12) and by Hyre (14).

One flask was subjected to all treatments except passage of the airstream through its solution. Titration of a 100 cc aliquot from this flask gave the exposure factor, i.e., that amount of carbon dioxide unavoidably absorbed by the solution in each flask during manipulation prior to and following a run.

Six towers were used for the leaf determinations, returning data on three pairs of leaves. Two towers, continuously connected to individual wet test meters, were employed in the measurement of the carbon dioxide content of the greenhouse air.

The first photosynthesis and respiration determinations were made

at the first opportunity after all the experimental infestations were established. From that point on, the leaves were checked at intervals which were about 10 days in length. The three test leaves of each mite population level, with their corresponding check leaves, were subjected to a determination on the same day. Morning runs were usually begun at about 8:00 a.m. and ended at about 12 noon, a duration of 4 hours. New flasks were then attached and the afternoon run started at about 12:30 p.m. and continued until about 4:30 p.m. for another 4-hour period. The leaves were retained on the cups and necessary titrations and preparations were made prior to the respiration run, which began at about 8:30 p.m. and continued until about 12:30 a.m. The exposed flasks were returned to the laboratory where they were made ready for titration on the following morning.

Effect on transpiration. The transpiration rates of the check and test leaves were measured during photosynthesis and respiration trials by placing drying tubes, charged with 4-mesh calicum chloride, ahead of and behind the cups. The first tube removed the greatest portion of the atmospheric moisture from the airstream, while the second tube removed the small amount of remaining airstream moisture plus the moisture given off by the leaves.

The difference in weight between the tubes attached to healthy and injured leaves was attributed to the unbalanced transpiration of the injured leaves. The small amount of atmospheric moisture remaining in the airstream after passage through the first drying tube was discounted since it was practically the same in all tubes.

Effect on chlorophyll content of leaves. A modification of the method of Compton and Boynton (6) was used to measure the chlorophyll content of the injured and healthy leaves. Twenty-five discs, comprising a total surface area of 64.33 cm^2, were cut from the leaves with a #9 cork borer, placed in 20 cc of 95 per cent methyl alcohol in a 2-ounce jar, and the chlorophyll extracted by leaving the jar in the dark for 24 hours. The extraction was then gravity filtered on S & S #595 paper, made up to 50 cc in volumetric flasks with fresh methyl alcohol, and subjected to colorimetric analysis. Experiments indicated that about 95 per cent of the available chlorophyll was removed from the leaf material by the first extraction. Leaf tissue was not macerated.

A reference curve was established by extracting enough leaf material to give a light transmission value of about 15 per cent. Two 100 cc samples were analyzed for chlorophyll content by the official AOAC method.[4] The remaining extractions were made into serial dilutions and

[4] Official Methods of Analysis of the Association of Official Agricultural Chemists, 8th Edition, 1955.

subjected to light transmission determinations on a Fischer Model AC electrophotometer. By calculation, using the chlorophyll content of the analyzed samples as a base point, a graph was set up for these data. Subsequent colorimetric determinations of chlorophyll extractions of injured and healthy leaves were translated into actual chlorophyll content by reference to the established curve.

The chlorophyll content of test and check leaves was measured on six dates; July 1, 15, and 25, August 4 and 14, and September 10. The sample leaves were brought into the laboratory and leaf punches were taken immediately after the numbers of mites per leaf were counted. The leaf punches were immersed in the methyl alcohol, put into a light-proof box, and held in the laboratory for the required 24-hour period. The extractions were processed the following day in semidarkness and the light transmission readings taken.

CLIMATIC MEASUREMENTS

Temperature and relative humidity were recorded automatically with a clock hygrothermograph and were also checked with a sling psychrometer. In addition, temperatures were read from a maximum-minimum thermometer. Further observations disclosed, however, that the readings obtained from the hygrothermograph were accurate enough to be used alone, and the supplementary measurements were omitted.

During the early part of the investigation, light readings were taken during the photosynthesis determinations. These were made with a General Electric Type PR-1 exposure meter, using the incident light attachment furnished with the meter. Readings were translated into foot candles by reference to tables supplied with the meter by the manufacturer. In spite of the fact that the greenhouse roof was coated with a mixture of hydrated spray lime and water, these readings showed that on clear, sunny days, the light values inside the house were well above the 1200-foot candles given by Heinicke and Hoffman (12) as the minimum light value at which apple leaves can be expected to assimilate carbon dioxide at their maximum rates. These readings also disclosed that on a given sunny day, these light values varied but slightly from those observed on other comparable days. Since the determinations were performed only on such days, it seemed logical to discontinue the light readings for the remainder of the study.

RESULTS

Behavior of the mite population. Once they were established, the experimental infestations required little attention. Transferred mites began feeding almost immediately and egg deposition was observed

shortly after transfer. In the low population groups, females lost in the first 3 days were replaced to maintain the relationship between the groups.

The time required for completion of development from egg to egg averaged about 16 days and egg hatch occurred about 6 days after deposition. These observations were made on separate leaves fitted with celluloid barriers (tables 1 and 2).

TABLE 1. Number of Days Between Generations of *M. ulmi* on Greenhouse-grown Red Delicious Leaves, 1956

Generations	Number of days	
	Trial 1	Trial 2
2-3	16	16
3-4	17	16
4-5	15	14
5-6	16	16
Totals	64	62
Averages	16	15.5

TABLE 2. Appearance Dates of Generations of *M. ulmi* on Greenhouse-grown Red Delicious Leaves, 1956*

Generation	Population groups					
	S1G1	S1G2	S1G3	S1G4	S2G1	S2G2
1†	June 7	June 10	June 14	June 20	June 24	June 28
2	" 23	" 26	" 30	July 6	July 10	July 14
3	July 9	July 12	July 16	" 22	" 26	" 30
4	" 25	" 28	Aug. 1	Aug. 7	Aug. 11	Aug. 15
5	Aug. 10	Aug. 13	" 17	" 23	" 27	" 31
6	" 26	" 29	Sept. 2	Sept. 8	Sept. 12	Sept. 16

* Calculated on calendar basis using average of table 1.
† Initial infestation regarded as first generation.

Injury was not confined to one surface and quiescent stages were found on both surfaces although egg deposition was considerably heavier on the under surface. Initial feeding seemed more severe on the apical and basal leaf areas.

When feeding injury reached the point where bronzing was evident over the entire leaf, a quick population decline took place. It was thought that perhaps debris and dust on these leaves prevented larval feeding and that mites in this stage died out. This was not the case, however, for such leaves when washed clean of the refuse, again failed to support newly added populations. The point at which this decline took place can be seen by comparing the data in tables 1 and 3.

Effect of the injury on photosynthesis. Substantial reductions were encountered in the photosynthesis rates of the various test leaves. These reductions were recorded quite early in the case of the dense population

groups, in sharp contrast to the relatively lengthy period of feeding before appreciable differences were noted in the low population groups.

Listed in tables 4 to 9 are the data obtained from the photosynthesis determinations. The analysis of variance of these data appears in table 10. Explanation of the probable causes of variations will be offered in the discussion.

In computing the analysis of variance, the values from the ratio trials were not included. Only those data obtained after feeding had been initiated were used.

Data listed under the heading "Per Cent of Expected Rate" are the result of dividing each successive T/C x 100 value by the average T/C x 100 value obtained from the first four determinations. Marshall, Childers and Brody (18) give this formula as follows:

$$\frac{\frac{\text{Test after treatment}}{\text{Check after treatment}}}{\frac{\text{Test before treatment}}{\text{Check before treatment}}} \times 100 = \text{Per cent expected rate}$$

TABLE 3. Mites per Greenhouse-grown Red Delicious Leaf, 1956

Date	Sample	Population group					
		S1G1	S1G2	S1G3	S1G4	S2G1	S2G2
July 1	A	13	33	74	137	84	83
	B	3	54	93	165	63	116
	C	7	21	67	143	107	90
	Av.	7.7	36.0	78.0	148.3	84.7	96.3
July 15	A	69	92	190	284	138	220
	B	108	107	207	302	103	384
	C	73	67	174	242	169	340
	Av.	83.3	88.8	190.3	279.3	136.7	314.7
July 25	A	173	220	311	469	312	473
	B	248	316	184	518	274	548
	C	190	163	280	316	429	497
	Av.	203.7	233.0	258.3	434.3	338.3	506.0
August 4	A	—	238	593	408	544	370
	B	—	297	374	486	620	246
	C	—	215	488	622	682	302
	Av.	—	250.0	488.3	472.0	615.3	306.0
August 14	A	412	522	306	112	190	148
	B	397	486	194	148	244	194
	C	360	561	240	94	152	122
	Av.	389.7	523.0	246.7	118.0	195.3	154.7
September 10	A	217	115	72	49	38	9
	B	320	84	50	55	64	24
	C	174	160	37	60	40	30
	Av.	237.0	119.7	53.0	54.7	47.3	21.0

Photosynthesis was generally higher during the morning hours than it was during the afternoon.

Effect of the injury on respiration. Determinations of respiratory activity were unsuccessful. The amount of carbon dioxide evolved by

TABLE 4. Photosynthesis of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 1, Group 1

Date		Mg CO_2 per liter of air	Mg CO_2 per hr./100 cm^2		T/C x 100	Per cent expected rate
			Check[1]	Test[1]		
June 5	am	.6384	9.53	10.07	105.7	
	pm	.6021	8.37	8.63	103.1	
June 6	am	.6742	12.00	14.90	124.2	
	pm	.6512	11.10	13.70	123.4	
				Av.	114.2	
June 29	am	.7210	8.28	8.20	99.0	86.7
	pm	.7057	13.02	14.32	110.0	96.3
				Av.	104.5	91.5
July 18	am	.5090	14.47	16.03	110.8	97.0
	pm	.4662	12.72	12.67	99.6	87.2
				Av.	105.2	92.1
July 30	am	.6226	10.96	13.41	122.4	107.2
	pm	.6170	10.47	12.20	116.5	102.0
				Av.	119.4	104.6
August 9	am	.4267	11.70	9.83	84.0	73.6
	pm	.4081	8.44	9.08	107.6	94.2
				Av.	95.8	83.9
September 15	am	.5945	6.58	5.44	82.7	72.4
	pm	.5608	5.25	4.00	76.2	66.7
				Av.	79.4	69.6

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	59	—		
Dates	4	15.53**	2.56	3.74
Treatments	1	—		
Replicates	2	5.38**	3.19	5.08
Treatment vs. dates	4	—		
Error	48	—		

** Significant at 1% level.

respiring leaves was apparently too small to be detected with any consistent accuracy by the equipment used in the study. The last determinations indicated a trend, but the degree of difference was not great enough to warrant inclusion in this report.

Effect of the injury on transpiration. Significant reductions in the transpiration rates of the injured test leaves were recorded during the study. An interesting development was that in all but two groups, S1G4 and S2G1, the transpiration rates of the test leaves showed a reduction at the outset which was followed by an increase in activity at about the mid-point of the study. Beyond this, the rates fell to rather low levels. In the two groups mentioned above, the decrease in transpiration was progressive with no increase in activity at mid-point as found in the other four groups.

TABLE 5. Photosynthesis of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 1, Group 2

Date		Mg CO_2 per liter of air	Mg CO_2 per hr./100 cm^2		T/C x 100	Per cent expected rate
			Check[1]	Test[1]		
June 8	am	.7145	14.83	15.93	107.4	
	pm	.6984	13.17	13.23	100.5	
June 9	am	.7402	15.53	15.13	97.4	
	pm	.7137	15.13	15.70	103.8	
				Av.	102.3	
June 30	am	.7255	13.67	15.73	115.1	112.5
	pm	.6713	17.97	15.67	87.2	85.2
				Av.	101.2	98.8
July 10	am	.5181	10.81	10.66	98.6	96.4
	pm	.6941	13.20	11.70	88.6	86.6
				Av.	93.6	91.5
July 21	am	.5285	12.81	10.87	84.9	83.0
	pm	.4971	11.89	11.67	98.1	95.9
				Av.	91.5	89.4
August 1	am	.7286	16.18	16.92	104.6	102.2
	pm	.6415	10.59	10.00	94.4	92.3
				Av.	99.5	97.2
August 11	am	.4570	14.24	11.08	77.8	76.1
	pm	.4398	13.65	9.70	71.1	69.5
				Av.	74.4	72.8
September 10	am	.4928	6.11	2.91	47.6	46.5
	pm	.4398	3.80	4.80	126.3	123.5
				Av.	87.0	85.0

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	71	—		
Dates	5	16.46**	2.65	3.53
Treatments	1	—		
Replicates	2	—		
Treatment vs. dates	5	—		
Error	58	—		

** Significant at the 1% level.

Unlike the photosynthesis determinations, the decreases in transpiration corresponded to the level of population in each group. Leaves bearing the highest population groups experienced the lowest transpiration rates at the conclusion of the study.

Data from these determinations were subjected to statistical treatment identical to that used for photosynthesis. Values from the first four determinations were not included in the analysis of variance of check and test.

The data are listed in tables 11 to 16, with individual group statistical analyses and with a summary on analysis of variance in table 17.

TABLE 6. Photosynthesis of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 1, Group 3

Date		Mg CO_2 per liter of air	Mg CO_2 per hr./100 cm^2		T/C x 100	Per cent expected rate
			Check[1]	Test[1]		
June 12	am	.6860	16.10	15.17	94.2	
	pm	.6493	13.77	14.40	104.6	
June 13	am	.7117	13.80	12.90	93.5	
	pm	.6551	12.77	11.17	87.5	
				Av.	95.0	
July 1	am	.6742	16.66	16.88	101.3	106.6
	pm	.6412	14.94	14.54	97.3	102.4
				Av.	99.3	104.5
July 13	am	.4980	15.96	15.58	97.6	102.7
	pm	.4220	13.51	12.12	89.7	94.4
				Av.	93.6	98.6
July 28	am	.4536	14.31	13.27	92.7	97.6
	pm	.4478	10 19	7.68	75.4	79.4
				Av.	84.0	88.5
August 7	am	.4091	15.57	9.41	60.4	63.6
	pm	.3847	11.01	8.14	73.9	77.8
				Av.	67.2	70.7
August 16	am	.5370	9.21	6.04	65.6	69.1
	pm	.4760	7.11	4.19	58.9	62.0
				Av.	62.2	65.6

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	59	—		
Dates	4	17.33**	2.56	3.74
Treatments	1	7.46**	4.04	7.19
Replicates	2	11.60**	3.19	5.08
Treatment vs. dates	4	—		
Error	48	—		

** Significant at the 1% level.

Effect of the injury on chlorophyll content of leaves. The reduction of the chlorophyll content of the test leaves can be seen by referring to the data in table 18. It may be noted that the reductions were slow starting in the low population groups as compared to the rapid depletion which took place at the higher population levels. Comparison of values for individual dates as listed in tables 3 and 18 will give the relationship between the mites present and the reduction in the chlorophyll content of that leaf.

It should be remembered that each sample value in tables 3 and 18 is related only in respect to sample source to the other five samples bearing the same letter for that particular date. Each replicate sample within a group was obtained from a different tree and, on any given date, that

Table 7. Photosynthesis of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 1, Group 4

Date		Mg CO_2 per liter of air	Mg CO_2 per hr./100 cm^2		T/C x 100	Per cent expected rate
			Check[1]	Test[1]		
June 18	am	.8090	17.07	17.93	105.0	
	pm	.7383	16.10	16.20	100.6	
June 19	am	.7125	17.20	14.20	82.6	
	pm	.6457	15.67	15.43	98.5	
				Av.	96.7	
July 2	am	.8435	16.27	17.21	105.8	109.4
	pm	.8164	14.61	10.87	74.4	76.9
				Av.	90.1	93.2
July 15	am	.6451	15.24	16.17	106.1	109.7
	pm	.6372	13.45	9.67	71.9	74.4
				Av.	89.0	92.0
July 24	am	.5309	12.64	9.28	73.4	75.9
	pm	.4929	14.40	10.96	76.1	78.7
				Av.	74.8	77.3
August 3	am	.5267	15.43	12.50	81.0	83.8
	pm	.4866	12.52	7.22	57.7	59.7
				Av.	69.4	71.8
August 13	am	.4813	10.33	7.22	69.9	72.3
	pm	.4640	6.61	3.51	53.1	54.9
				Av.	61.5	63.6

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	59	—		
Dates	4	8.59**	2.56	3.74
Treatments	1	8.67**	4.04	7.19
Replicates	2	—		
Treatment vs. dates	4	—		
Error	48	—		

** Significant at the 1% level.

sample has only a time and population level relationship with the other two samples in that group.

A point of interest is the fact that it was not possible to obtain a reduction of the chlorophyll content of the test leaves lower than an average of 41 per cent. Although this is a severe depletion of the chlorophyll in a leaf, it by no means compares with the almost complete removal of chlorophyll by the mite as reported by other workers. There will be some further discussion on this point.

TABLE 8 Photosynthesis of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 2, Group 1

Date		Mg CO_2 per liter of air	Mg CO_2 per hr./100 cm^2		T/C x 100	Per cent expected rate
			Check[1]	Test[1]		
June 22	am	.6946	14.17	14.23	100.4	
	pm	.6781	13.47	12.40	92.1	
June 23	am	.7109	14.43	15.13	104.9	
	pm	.6870	17.83	14.22	79.8	
				Av.	94.3	
July 3	am	.7119	16.24	16.28	100.2	106.3
	pm	.6635	13.11	11.95	91.2	96.7
				Av.	95.7	101.5
July 16	am	.7028	16.08	15.57	96.8	102.7
	pm	.6754	14.16	11.43	80.7	85.6
				Av.	88.8	94.2
August 4	am	.4771	11.75	7.22	61.4	65.1
	pm	.4349	7.30	6.05	82.9	87.9
				Av.	72.2	76.5
August 14	am	.4975	12.76	7.04	55.2	58.5
	pm	.4680	5.64	4.70	83.3	88.3
				Av.	69.2	73.4
September 5	am	.6372	4.06	2.05	50.5	53.6
	pm	.6110	2.39	1.08	45.2	47.9
				Av.	47.8	50.8

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	71	—		
Dates	5	36.40**	2.38	3.37
Treatments	1	10.57**	4.02	7.12
Replicates	2	—		
Treatment vs. dates	5	—		
Error	58	—		

** Significant at the 1% level.

TABLE 9. Photosynthesis of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 2, Group 2

Date		Mg CO_2 per liter of air	Mg CO_2 per hr./100 cm^2		T/C x 100	Per cent expected rate
			Check[1]	Test[1]		
June 26	am	.5747	11.91	11.23	94.3	
	pm	.5363	14.59	15.41	105.6	
June 27	am	.6146	18.53	18.02	97.2	
	pm	.5727	14.36	13.67	95.2	
				Av.	98.1	
July 5	am	.5142	16.61	14.40	86.7	88.4
	pm	.4734	12.76	12.28	96.2	98.1
				Av.	91.4	93.2
July 17	am	.5141	19.19	17.06	88.9	90.6
	pm	.4375	6.35	4.77	75.1	76.6
				Av.	82.0	83.6
July 26	am	.4329	13.97	8.99	64.4	65.6
	pm	.4015	9.64	6.30	65.4	66.7
				Av.	64.9	66.2
August 6	am	.3984	12.27	9.43	76.9	78.4
	pm	.3751	11.89	7.81	65.7	67.0
				Av.	71.3	72.7
August 15	am	.5174	9.26	5.36	57.9	59.0
	pm	.5008	4.68	2.76	59.0	60.1
				Av.	58.4	59.6
September 3	am	.6140	4.44	2.34	52.7	53.7
	pm	.5347	2.40	1.55	64.6	65.9
				Av.	58.6	59.8

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	71	—		
Dates	5	14.10**	2.38	3.37
Treatments	1	7.88**	4.02	7.12
Replicates	2	—		
Treatment vs. dates	5	—		
Error	58	—		

** Significant at the 1% level.

TABLE 10. Analysis of Variance of Photosynthesis of Groups and Treatments

Source	d.f.	Sum of squares	Mean squares	F	5%	1%
Total	395	9351.37	23.66	—		
Treatment within groups	6	385.71	64.28	2.92**	2.14	2.90
Groups	6	521.26	86.88	3.94**	2.14	2.90
Error	383	8444.40	22.05	—		

** Significant at the 1% level.

TABLE 11. Transpiration of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 1, Group 1

Date		Grams H_2O per hour/100 cm^2		T/C x 100	Per cent expected rate
		Check[1]	Test[1]		
June 5	am	1.36	1.33	97.8	
	pm	1.59	1.58	99.4	
June 6	am	1.69	1.66	98.2	
	pm	1.46	1.44	98.6	
				Av. 98.5	
June 29	am	1.40	1.35	96.4	97.9
	pm	1.52	1.46	96.1	97.6
				Av. 96.2	97.8
July 18	am	1.27	1.36	107.1	108.7
	pm	1.25	1.14	91.2	92.6
				Av. 99.2	100.6
August 9	am	1.22	1.30	106.6	108.2
	pm	1.45	1.66	114.5	116.2
				Av. 110.6	112.2
August 15	am	1.21	.94	77.7	78.9
	pm	1.13	1.11	98.2	99.7
				Av. 88.0	89.3

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	59	—		
Dates	4	9.37**	2.56	3.74
Treatments	1	—		
Replicates	2	10.83**	3.19	5.08
Treatment vs. dates	4	—		
Error	48	—		

** Significant at the 1% level.

TABLE 12. Transpiration of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 1, Group 2

Date		Grams H_2O per hour/100 cm^2		T/C x 100	Per cent expected rate
		Check[1]	Test[1]		
June 8	am	1.52	1.61	105.9	
	pm	1.53	1.63	106.5	
June 9	am	1.64	1.65	100.6	
	pm	1.60	1.79	111.9	
			Av.	106.2	
June 30	am	1.43	1.69	118.2	111.3
	pm	1.77	1.78	100.6	94.7
			Av.	109.4	103.0
July 10	am	1.69	1.75	103.6	97.6
	pm	1.50	1.63	108.7	102.4
			Av.	106.2	100.0
July 21	am	1.30	1.37	105.4	99.2
	pm	1.57	1.90	121.0	113.9
			Av.	113.2	106.6
August 1	am	1.56	1.62	103.2	97.2
	pm	1.43	1.45	101.4	95.5
			Av.	102.3	96.4
August 11	am	1.22	1.13	92.6	87.2
	pm	1.46	1.26	86.3	81.3
			Av.	89.4	84.2
September 10	am	1.47	1.07	73.3	69.0
	pm	1.58	1.21	76.6	72.1
			Av.	75.0	70.6

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	71	—		
Dates	5	6.72**	2.38	3.37
Treatments	1	—		
Replicates	2	—		
Treatment vs. dates	5	—		
Error	58	—		

** Significant at the 1% level.

TABLE 13. Transpiration of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956
Series 1, Group 3

Date		Grams H_2O per hour/100 cm^2		T/C x 100	Per cent expected rate
		Check[1]	Test[1]		
June 12	am	1.44	1.53	106.2	
	pm	1.30	1.39	106.9	
June 13	am	1.24	1.23	99.2	
	pm	1.15	1.19	103.5	
				Av. 104.0	
July 1	am	1.81	1.62	89.5	86.1
	pm	1.78	1.35	75.8	72.9
				Av. 82.6	79.5
July 13	am	1.29	1.45	112.4	108.1
	pm	1.47	1.56	106.1	102.0
				Av. 109.2	105.0
July 28	am	1.26	1.47	116.7	112.2
	pm	1.54	1.40	90.9	87.4
				Av. 103.8	99.8
August 7	am	1.26	1.30	103.2	99.0
	pm	1.48	1.52	102.7	99.0
				Av. 103.0	99.0
August 16	am	1.45	1.37	94.5	90.9
	pm	1.86	1.17	62.9	60.5
				Av. 78.7	75.7

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	59	—		
Dates	4	—		
Treatments	1	—		
Replicates	2	5.73**	3.19	5.08
Treatment vs. dates	4	—		
Error	48	—		

** Significant at the 1% level.

TABLE 14. Transpiration of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956
Series 1, Group 4

Date		Grams H_2O per hour/100 cm^2		T/C x 100	Per cent expected rate
		Check[1]	Test[1]		
June 18	am	1.80	1.98	110.0	
	pm	1.31	1.53	116.8	
June 19	am	1.45	1.33	91.7	
	pm	1.46	1.51	103.4	
				Av. 105.5	
July 2	am	1.58	1.97	124.7	118.2
	pm	1.35	1.59	117.8	111.7
				Av. 121.2	115.0
July 15	am	1.43	1.39	97.2	92.1
	pm	1.49	1.36	91.3	86.5
				Av. 94.2	89.3
July 24	am	1.17	1.12	95.7	90.7
	pm	1.41	1.30	92.2	87.4
				Av. 94.0	89.0
August 3	am	.92	.71	77.2	73.2
	pm	1.27	.75	59.1	56.0
				Av. 68.2	64.6
August 13	am	1.06	.91	85.8	81.3
	pm	1.65	1.13	68.5	64.9
				Av. 77.2	73.1

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	59	—		
Dates	4	7.33**	2.56	3.74
Treatments	1	—		
Replicates	2	—		
Treatment vs. dates	4	—		
Error	48	—		

** Significant at the 1% level.

TABLE 15. Transpiration of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 2, Group 1

Date		Grams H_2O per hour/100 cm^2		T/C x 100	Per cent expected rate
		Check[1]	Test[1]		
June 22	am	1.39	1.50	107.9	
	pm	1.40	1.42	101.4	
June 23	am	1.72	1.57	91.3	
	pm	1.61	1.68	104.3	
				Av. 101.2	
July 3	am	1.60	1.51	93.8	92.7
	pm	1.17	1.34	114.5	113.1
				Av. 104.2	102.9
July 16	am	1.19	1.58	132.8	131.2
	pm	1.46	1.04	71.2	70.4
				Av. 102.0	100.8
July 25	am	1.37	1.16	84.7	83.7
	pm	1.48	1.53	103.4	102.2
				Av. 94.0	93.0
August 4	am	1.05	.82	78.1	77.2
	pm	1.40	1.23	87.9	86.9
				Av. 83.0	82.0
August 14	am	.75	.43	57.3	56.6
	pm	.95	.69	72.6	71.7
				Av. 65.0	64.2
September 5	am	1.21	.86	71.1	70.3
	pm	1.20	.70	58.3	57.6
				Av. 64.7	64.0

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	71	—		
Dates	5	13.09**	2.38	3.37
Treatments	1	7.06*	4.02	7.12
Replicates	2	—		
Treatment vs. dates	5	—		
Error	59	—		

** Significant at 1% level.
* Significant at 5% level.

TABLE 16. Transpiration of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Series 2, Group 2

Date		Grams H_2O per hour/100 cm^2		T/C x 100	Per cent expected rate
		Check[1]	Test[1]		
June 26	am	1.47	1.57	106.8	
	pm	1.76	1.73	98.3	
June 27	am	1.40	1.42	101.4	
	pm	1.58	1.35	85.4	
			Av.	98.0	
July 5	am	1.57	1.70	107.6	109.8
	pm	1.39	1.20	86.3	88.1
			Av.	97.0	99.0
July 17	am	1.68	1.62	96.4	98.4
	pm	1.72	1.49	86.6	88.4
			Av.	91.5	93.4
July 26	am	1.20	1.15	95.8	97.8
	pm	1.54	1.48	96.1	98.1
			Av.	96.0	98.0
August 6	am	1.14	.71	62.3	63.6
	pm	1.31	.83	63.4	64.7
			Av.	62.8	64.2
August 15	am	.83	.54	65.1	66.4
	pm	1.15	.71	61.7	63.0
			Av.	63.4	64.7
September 3	am	.92	.52	56.5	57.7
	pm	1.10	.48	43.6	44.5
			Av.	50.0	51.1

[1] Average value for 3 leaves.

Analysis of Variance of Check and Test

Source	d.f.	F	5%	1%
Total	71	—		
Dates	5	22.86**	2.38	3.37
Treatments	1	17.14**	4.02	7.12
Replicates	2	—		
Treatment vs. dates	5	—		
Error	58	—		

** Significant at the 1% level.

TABLE 17. Analysis of Variance of Transpiration of Groups and Treatments.

Source	d.f.	Sum of squares	Mean squares	F	5%	1%
Total	395	59.77	.151	—		
Treatment within groups	6	9.25	1.54	13.63**	2.14	2.90
Groups	6	7.20	1.20	10.62**	2.14	2.90
Error	383	43.32	.113	—		

** Significant at the 1% level.

DISCUSSION

Behavior of the mite populations. The elapsed time between generations in this study was somewhat longer than that reported by Cagle (3) and Garman (8). At an average temperature of 71.4° F., the incubation period of about six days compared favorably with the 7-day period given by Cagle and the 6-day period reported by Garman. However, the time required for the mites to develop into adults after eclosion was about ten days, a considerably longer period than the 6 to 7 days observed by the above authors. Availability of food was adequate at all times since transfers were made to healthy leaf tissue.

It should be noted that although the mite counts listed in table 3 follow a relatively uniform progression, there may be variations present which, while not readily visible, are perhaps of some importance. Since each mite count was made on a leaf bearing a discrete population that was established early in the study, the rise and decline of that particular population up to the time the count was made may be somewhat different than the information in the table might indicate. If the counts had been made on the same leaves on each of the five dates, the possibility exists that the populations could have followed a different trend from that indicated by the numbers in table 3. The fact that each count was made on leaves which were subsequently destroyed in the chlorophyll analyses made such individual populations a necessity.

TABLE 18. Chlorophyll Content of Greenhouse-grown Red Delicious Leaves Injured by *M. ulmi* Feeding, 1956

Date	Sample	Mg/100 cm² Test	Mg/100 cm² Check	% red.	Mg/100 cm² Test	Mg/100 cm² Check	% red.
		Group S1G1			Group S1G2		
July 1	A	3.13	3.13	0.00	3.12	3.12	0.00
	B	3.07	3.09	0.65	3.23	3.23	0.00
	C	3.12	3.13	0.32	3.08	3.14	1.91
	Av.			0.32			0.64
July 15	A	3.08	3.08	0.00	2.94	3.04	3.29
	B	3.00	3.04	1.32	2.90	3.07	5.54
	C	3.08	3.12	1.28	3.00	3.14	4.45
	Av.			0.87			4.43
July 25	A	2.86	3.00	4.67	2.68	3.07	12.70
	B	3.23	3.37	4.15	2.65	2.94	9.86
	C	3.14	3.31	5.14	2.80	3.14	10.83
	Av.			4.65			11.13
August 4	A				2.39	3.14	23.89
	B				2.49	3.14	20.70
	C				2.22	3.04	26.97
	Av.						23.85
August 14	A	2.39	3.08	22.40	2.36	3.07	23.13
	B	2.56	3.19	19.75	2.42	3.23	25.08
	C	2.52	3.14	19.75	2.39	3.07	22.15
	Av.			20.63			23.45

TABLE 18.—Concluded

Date	Sample	Mg/100 cm² Test	Mg/100 cm² Check	% red.	Mg/100 cm² Test	Mg/100 cm² Check	% red.
September 10	A	2.16	3.08	29.87	1.85	2.94	37.07
	B	2.16	3.12	30.77	2.03	3.16	35.76
	C	2.24	3.08	27.27	1.99	3.08	35.39
	Av.			29.30			36.07
		Group S1G3			**Group S1G4**		
July 1	A	2.94	3.08	4.55	2.85	3.14	9.24
	B	3.23	3.31	2.42	2.90	3.08	5.84
	C	2.96	3.12	5.13	2.74	3.14	12.74
	Av.			4.03			9.27
July 15	A	2.74	3.00	8.67	2.56	3.04	15.79
	B	2.65	3.04	12.83	2.83	3.19	11.29
	C	2.83	3.14	9.87	2.42	3.00	19.33
	Av.			10.46			15.47
July 25	A	2.86	3.51	18.52	2.52	3.23	21.67
	B	2.39	3.04	21.38	2.21	3.12	29.17
	C	2.53	3.00	15.67	2.44	3.33	26.73
	Av.			18.52			25.86
August 4	A	2.18	3.00	27.33	1.90	3.14	39.49
	B	2.03	2.94	30.95	2.06	3.04	32.24
	C	2.07	3.04	31.91	2.10	3.37	37.69
	Av.			30.06			36.47
August 14	A	1.82	3.04	40.13	1.92	3.19	39.81
	B	1.89	3.19	40.75	1.81	2.90	37.59
	C	1.97	3.23	39.01	2.18	2.96	26.35
	Av.			39.96			34.58
September 10	A	1.92	3.16	39.24	1.79	2.88	37.85
	B	1.96	3.27	40.06	1.83	2.70	32.22
	C	2.24	3.08	27.27	2.10	3.27	35.78
	Av.			35.53			35.28
		Group S2G1			**Group S2G2**		
July 1	A	3.04	3.16	3.80	3.04	3.33	8.71
	B	3.08	3.43	10.20	2.65	3.04	12.82
	C	2.77	3.08	10.07	3.08	3.37	8.61
	Av.			8.02			10.05
July 15	A	2.53	3.04	16.78	2.47	3.14	21.34
	B	2.49	3.33	25.23	2.32	3.19	27.27
	C	2.32	3.12	25.64	2.61	3.43	23.91
	Av.			22.55			24.17
July 25	A	2.30	3.16	27.22	2.21	3.23	31.58
	B	2.39	3.08	22.40	2.24	3.08	27.27
	C	2.00	3.31	39.58	1.97	3.00	34.33
	Av.			29.73			31.06
August 4	A	2.28	3.33	31.53	2.16	3.31	34.74
	B	2.03	3.23	37.15	1.94	3.04	36.18
	C	2.02	3.23	37.46	2.10	3.19	34.17
	Av.			35.38			35.03
August 14	A	1.77	2.83	37.32	2.02	3.08	34.42
	B	1.94	3.08	37.01	2.09	3.14	33.44
	C	2.09	3.12	33.01	2.10	3.04	30.92
	Av.			35.78			32.93
September 10	A	1.89	3.00	37.00	1.67	3.00	44.33
	B	1.72	3.23	46.75	1.76	2.80	37.14
	C	1.81	3.04	40.46	1.82	3.04	40.13
	Av.			41.40			40.53

Examination of the data in table 3 will reveal that in all but one case the decline in the number of mites in the separate groups was rapid. By comparing these data with those in table 18, it will be seen that decline began as the reduction in chlorophyll content of the leaves approached 35 per cent. Further discussion will be found in the section on chlorophyll reduction.

Effect of the injury on photosynthesis. As noted previously, variations are present in the results. According to Pickett (19), it is pointed out by authors that certain details must be carefully attended to if ac-

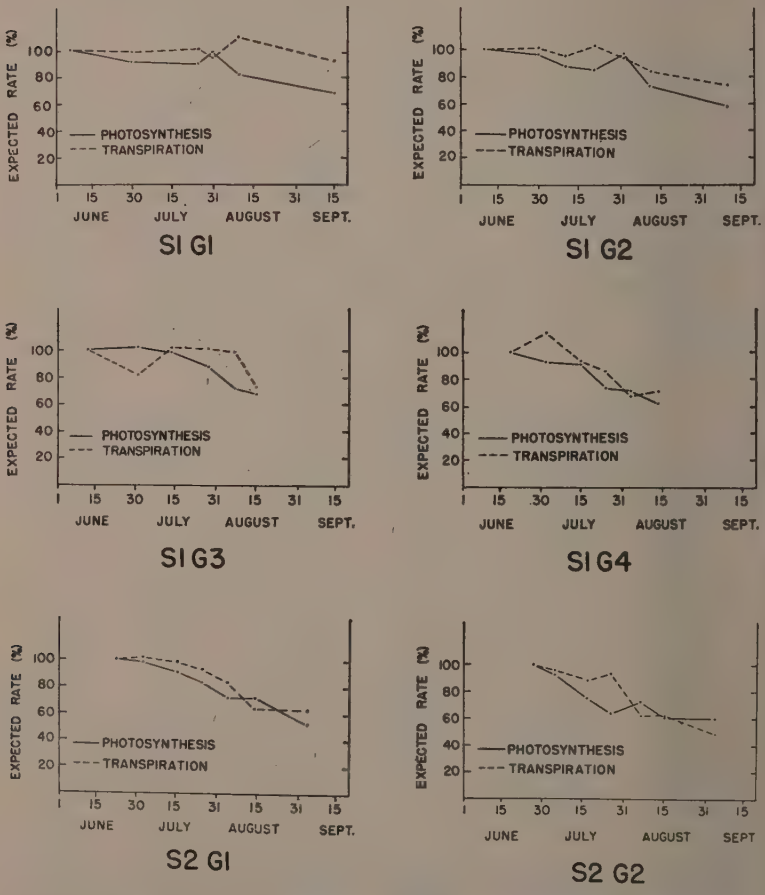

FIG. 1. Effect of *M ulmi* feeding injury on photosynthesis and transpiration of greenhouse-grown Red Delicious leaves, 1956.

curate results are to be realized in the measurements. Among these are: (1) the regulation of the airstream, (2) constant temperature of the airstream, (3) carbon dioxide content of the airstream, (4) regularity of the illumination, and (5) the use of apparatus suitable for the collection of the gas while placing the plant under conditions favorable for photosynthesis.

Use of the Heinicke-Hoffman apparatus, with individual cup chambers for each leaf, fulfills the demands of (5) above. The carbon dioxide content of the airstream (3), was relatively stable; any radical changes in concentration appeared to take place at night. The fact that the determinations were performed only on clear sunny days gives reason to discount (4). Factors (1) and (2) are left. It is obvious that the only

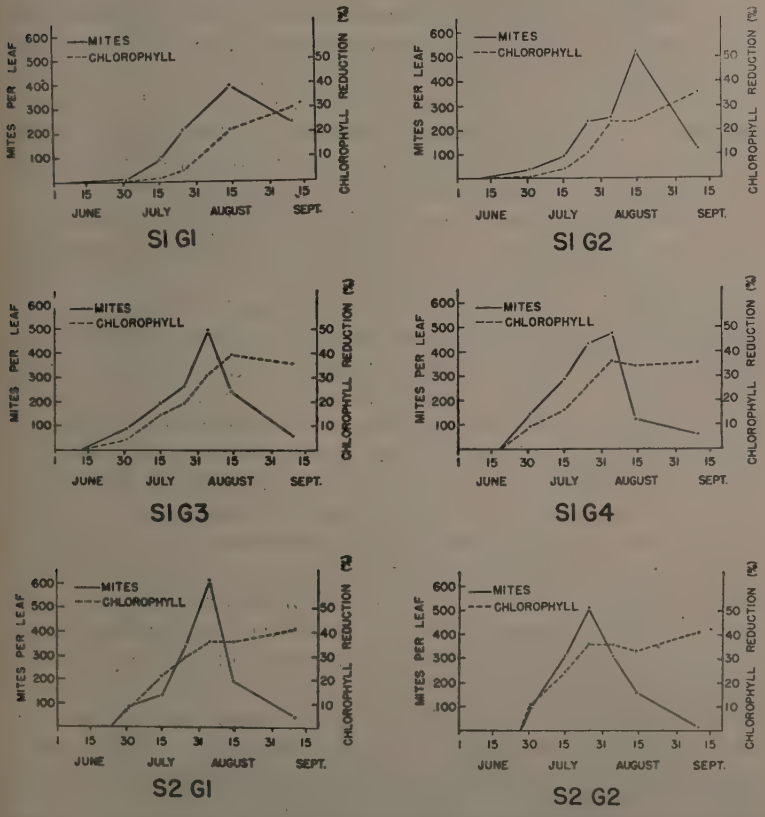

FIG. 2. Comparison of *M. ulmi* populations and per cent reduction of chlorophyll content of greenhouse-grown Red Delicious leaves, 1956.

solution to (2) is the use of a constant temperature chamber such as that employed by Childers and Brody (5).

Regulation of the airstream (1) appears to be the most critical factor of those listed. In personal communication with the writer, workers familiar with the method have indicated that, in their experience, dependability was achieved by the use of individual wet test meters for each absorption tower. The use of one meter to measure the air flow at the middle of the run produced variations which could not be eliminated. It is clear that fluctuations occurring before or after such measurement are lost to the observer. In this study, the air flow through the two check towers was recorded continuously by a wet test meter attached in each system during the entire period of each determination. The values recovered from these towers varied about 1 per cent from their average, a degree of accuracy which could not be equalled in the remaining towers where calibration of the mercury flowmeters was done at the middle of the run.

Another factor that may interfere with the accuracy of the determinations is decribed by Heinicke and Hoffman (12) as the difference in the activity of adjacent leaves on the same shoot. In 49 trials, these authors recorded a greater activity for the upper leaf of a pair in 53 per cent of the cases. In 22 per cent of the cases, the lower leaf was the more active, and in 25 per cent, activity was relatively even. Differences in activity ranged from 0 to 40 per cent.

Comparison of the data in tables 4 to 9 and in figure 1 with the reductions in chlorophyll in table 18 will reveal that, in several instances, the photosynthesis rate of a leaf group remained relatively high in spite of the fact that chlorophyll in other leaves bearing the same population level was at this time seriously depleted. The reader's attention is called to the values for groups S1G3 on July 1 and 13, S1G4 on July 2 and 15, and S2G1 on July 3 and 16. In all of these instances, the reduction in the rate of assimiliation is not proportional to reduction of chlorophyll for about the same dates. Marshall, Childers and Brody (18) found this to be the case in their experiments with leafhopper feeding injury on apple leaves. They attributed this to the fact that the spongy mesophyll of the leaf beneath injured palisade cells received more light than under normal circumstances and that an increased rate of photosynthesis was carried on in this region.

Hand sections of injured Red Delicious leaves made by the writer showed that in the early stages injury was principally in the uppermost palisade layers. Three distinct layers were visible in many of the sections. According to Pickett (19), leaves produced on potted trees in a greenhouse have less palisade development than leaves of the same variety

grown in the orchard. McDaniels and Cowart (17) state that the number of palisade layers may vary from one in shaded leaves and leaves at the base of vegetative shoots to three and possibly four layers on leaves of vigorously growing vegetative shoots. Observations in this study agree with those of the latter two authors. In such a situation, therefore, leaf reaction to early feeding would parallel that suggested by Marshall, Childers and Brody (18).

Review of the data in the statistical analysis tables discloses that the reduction of photosynthesis is highly significant with respect to dates in all groups. Since injury is progressive, such variation is to be expected over the course of the study. The fact that the F values for both dates and treatments do not increase proportionally, nor remain constant with the population levels, may be due as suggested earlier to differences in the numbers of mites actually present on these leaves as compared to the numbers which might be expected to result from the initial infestations.

Effect of the injury on transpiration. As indicated in tables 11 to 16, and in figure 2, the feeding injury exerted considerable effect on transpiration. Of interest is the slight rise following an initial decrease which took place in four of the six groups.

The over-all reduction in transpiration pointed to one clear fact: the more rapid reductions in chlorophyll and photosynthesis resulted in a lower transpiration rate in the final determinations. This may be seen by comparing the values for the last trials in groups S1G1 and S2G2, the lowest and highest population levels. These are 68.69, 90.56, and 60.01, 49.49, for the percentage of expected rate of photosynthesis and transpiration for each group respectively. The only apparent difference in external influence on the leaves of these groups is the rate of accumulation of the injury, i.e., where the damage was slowly progressing in group S1G1, group S2G2 suffered severe injury in a comparatively short period. Such intense feeding may disturb the physiological balance of the leaf more severely than the slower feeding.

Effect of the injury on the chlorophyll content of leaves. In spite of the dense populations present on the test leaves, it was not possible in any instance to obtain reductions in the chlorophyll content of these leaves below an average of 41 per cent.

This was surprising in view of reports in the literature where chlorophyll was purported to have been almost completely removed. On the other hand, Chapman, Lienk and Curtis (4), and Lienk, Chapman and Curtis (16) found that under orchard conditions, unchecked infestations of the European red mite and the two-spotted spider mite *(Tetranychus telarius L.)* reduced the chlorophyll content of Red Delicious leaves by only 34 per cent in 1951, and 36 per cent in 1952. Thus the reductions

recorded in this study would seem to agree more closely with the results of these authors than with the other reports mentioned above.

When reductions reached about 30 per cent in the high population groups, it became evident that the chlorophyll content of the leaves was approaching what might be termed a static condition. Depletion was still taking place but was extremely slow in comparison to the earlier rate. This may be seen by referring to figure 2. Since, as explained previously, decline in mite numbers and the retarded reduction of chlorophyll were apparently not due to debris on the leaves, several possible reasons were suggested as the cause of this decline. Among them were the following: (1) insufficient length of larval stylets to allow mites at this stage to penetrate to remaining healthy cells, and (2) increase in hardness and density of the cuticle and epidermal layer as the leaves reach a stage of maturity at which larval stylets may not be strong enough to penetrate them.

The amount of chlorophyll recovered from uninjured Red Delicious leaves grown in the greenhouse, although somewhat lower, compares favorably with that reported by Lienk, Chapman and Curtis (16) from uninjured orchard leaves of the same variety. These authors list values ranging from 2.7 to 3.9 mg per 100 cm^2 of leaf surface in comparison to values of 2.70 to 3.51 mg recovered by the writer from equal areas.

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